

Change of Ultraviolet Absorbance of Sunscreens by Exposure to Solar-Simulated Radiation

Harald Maier, Günther Schaubberger,* Konrad Brunnhofer,† and Herbert Hönigsmann

Division of Special and Environmental Dermatology, University of Vienna Medical School, Vienna, Austria; *Institute of Medical Physics and Biostatistics, University of Veterinary Medicine Vienna, Vienna, Austria; †Austrian Consumers' Association, Vienna, Austria

Regarding the outdoor behavior of the Caucasian population, modern sunscreens should provide high and broad-spectrum ultraviolet protection in the ultraviolet B as well as in the ultraviolet A range and should be photochemically stable for ultraviolet doses, which can be expected in solar radiation. At present an assessment of the photostability of suncare products is not a general requirement before marketing. In order to evaluate the photostability of suncare products we conducted an *in vitro* test and measured the spectral absorbance of 16 sunscreens before, and after exposure to increasing biologically weighted standard erythema doses (5, 12.5, 25, 50) of solar-simulated radiation. Seven of 16 suncare products showed a significant dose- and wavelength-dependent decrease of the ultraviolet A protective capacity, whereas the ability to absorb ultraviolet B was not affected. In the ultraviolet A range, the decrease of absorbance (photoinactivation), respectively, the

increase of transmission was 12–48% for an ultraviolet exposure of 25 standard erythema dose. Photoinactivation started in the wavelength range between 320 and 335 nm with a maximum above 350 nm. Furthermore, our analysis showed that the behavior of suncare products was not predictable from its individual ingredients. Neither complex combinations of organic filters nor addition of inorganic filters could absolutely prevent photoinactivation. The inclusion of a single photounstable filter did not mean photoinstability of the complete suncare product. Photoinactivation of sunscreens appears to be an underestimated hazard to the skin, first, by formation of free radicals, second, by increased ultraviolet A transmission. **Key words:** photoinstability/standard erythema dose/ultraviolet exposure/photodamage/solar radiation. *J Invest Dermatol* 117:256–262, 2001

Recent studies show that regular use of sunscreens reduces the carcinogenic risk (Thompson *et al*, 1993; Fourtanier, 1996), provides protection against immune suppression (Wolf *et al*, 1993; Krien and Moyal, 1994; Damian *et al*, 1997; Serre *et al*, 1997) and prevents photoaging (Harrison *et al*, 1991; Bernstein *et al*, 1997). It has been pointed out repeatedly that ultraviolet (UV) A radiation (320–380 nm) contributes to skin photodamage (Parrish *et al*, 1978). Photodamage is predicted to become a major threat to public health in the coming decades (Kaminer, 1995); therefore, in addition to a high protection in the UVB range (280–320 nm) also protection against UVA radiation appears to be mandatory (Young and Walker, 1999). A new generation of broad-spectrum sunscreens provides high protective capacity in both, the UVA and UVB range. The major advantage of these suncare products should be a more effective protection of acute and chronic adverse effects of solar UV radiation to skin areas usually exposed to

sunlight (face, hands, lower legs) and areas exposed under special conditions (outdoor work, outdoor sport). The misleading advertising of a “safe tan” by sunscreen producers, however, encourages people to (mis)use modern sunscreens in order to stay much longer in the sun (Boldeman *et al*, 1996; Autier *et al*, 1998). Prolonged exposure time together with a wrong self-assessment of the personal burning capacity (Stender *et al*, 1996) and incorrect mode of application (Azurdia *et al*, 1999; Pruijm *et al*, 1999) reduces the benefits of high protective factors and broad-spectrum protection. At present, it appears that in public opinion sunscreens are regarded as first-line photoprotective modality. This is the reason why an International Working Group of experts convened by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) concluded that sunscreens should be only one part of a comprehensive sun avoidance strategy (IARC, 2000a). The most important recommendations on sun behavior forwarded by the IARC are, moving into the shade, wearing UV protective clothing (IARC, 2000b) and that advertising should avoid promoting sunscreens for intentional sun exposure (IARC, 2000b). Furthermore, evidence is increasing that sunscreens may not be as harmless as they have been supposed to be (Knowland *et al*, 1997). Suncare products are a mixture of many different ingredients, all of them should resist UV doses relevant for a sunny day. It was shown, however, that certain organic UV filters are inactivated by UV radiation (Chignell *et al*, 1980; Gasparro, 1985; Knowland *et al*, 1993; Schwack and Rudolph, 1995; Allen *et al*, 1996; Berset *et al*, 1996; Schallreuter *et al*, 1996; Tarras-

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Reprint requests to: Dr. Harald Maier, Division of Special and Environmental Dermatology, University of Vienna Medical School, Währinger Gürtel 18–20, A-1090 Vienna, Austria.

Abbreviations: IARC, International Agency for Research on Cancer; ROS, reactive oxygen species; SED, standard erythema dose = 100 J per m² of erythemally weighted UV radiation; UVA, ultraviolet A = wavelength 320–380 nm; UVB, ultraviolet B = wavelength 280–320 nm.

Table I. Sunscreen active ingredients and declaration of photobiologically relevant data on package

Filter substances (chemical filters according to CTFA ^a /INCI ^b)	Sunscreen product ^h															
	A	B	C	D	E	F	G	H	I	K	L	M	N	O	P	Q
Zinc oxide								x								
Titanium dioxide	x	x		x	x		x		x	x	x	x	x	x	x	x
Octocrylene	x							x			x		x			
Benzophenone-3							x									
Phenylidibenzimidazole sulfonic acid			x				x									
Terephthalylidene dicamphor sulfonic acid	x										x		x			
Butyl methoxydibenzoylmethane	x	x	x	x	x	x			x	x	x	x	x	x	x	x
Mexoryl SX				x												
Isoamyl p-methoxycinnamate						x	x								x	
Octyl methoxycinnamate		x	x		x	x		x	x	x		x		x	x	x
Methylbenzylidene camphor		x		x	x	x	x		x	x	x	x		x	x	x
Octyl triazone			x		x	x				x				x		
Broad spectrum protection made evident	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SPF ^c	20	18	20	20	20	16	18	20	24	18	25	26 ^e	25	25	18	18
UVA protection factor/method of assessment			AUS ^d	7PPD ^e 18 IPD ^f	AUS			AUS		AUS		AUS	10 PPD	AUS	AUS	
Photostability made evident	x										x					

^aCosmetic Toiletry and Fragrance Association.^bInternational Nomenclature Cosmetic Ingredient.^cSun Protection Factor.^dAustralian Standard.^ePermanent Pigment Darkening.^fImmediate Pigment Darkening.^gIn all other European countries only available with SPF = 25.

^hSunscreen producers: A Ambre Solaire Intensivschutz Sonnenmilch (Laboratoires Garnier, Paris, France); B AS SUN Sonnenmilch (E. Kiessling & Cie GmbH & Co., Georgsmünd, Germany); C delial Sensitive Sonnen Balm (Bayer AG, Leverkusen, Germany); D Anthelios Sonnenmilch (La Roche-Posay Laboratoire Pharmaceutique, La Roche – Posay, France); E Nivea Sun Sonnenmilch (Beiersdorf AG, Hamburg, Germany); F Nivea Sun Sonnen Balsam (Beiersdorf AG, Hamburg, Germany); G Tiroler Nussöl Sensitiv Sonnen Milch (Tiroler Nussöl Sonnenkosmetik, München, Germany); H AS Sonnenmilch für Kinder (E. Kiessling & Cie GmbH & Co Georgsmünd, Germany); I Ellen Betrix Sun Care Sonnenmilch für Kinder (P & G, Weybridge, UK); K Nivea Sun Sonnenmilch für Kinder (Beiersdorf AG, Hamburg, Germany); L Ambre Solaire Kinder Intensivschutz Sonnenmilch (Laboratoires Garnier, Paris, France); M Delial Sonnenmilch für Kinder (Sara Lee, Düsseldorf, Germany); N Vichy Capital Soleil Sunblocker-Milch speziell für Kinderhaut (Vichy Laboratoires, Vichy, France); O pH5-Eucerin Sun Sensitive Kinder Lotio (Beiersdorf AG, Hamburg, Germany); P Bübchen Sonnen Milk (Bübchen Werk Ewald Hermes Pharmazeutische Fabrik GmbH, Soest, Germany); Q Penaten Baby Sonnenmilch (Johnson & Johnson GmbH, Düsseldorf, Germany).

Wahlberg *et al*, 1999; Vanquerp *et al*, 1999), lose their UV protective capacity (Diffey *et al*, 1997) and may behave as photo-oxidants (Allen *et al*, 1996; Schallreuter *et al*, 1996; McHugh and Knowland, 1997). This is of utmost significance because filter molecules penetrate through the epidermis (Kenney *et al*, 1995; Hayden *et al*, 1997; Jiang *et al*, 1999) and highly reactive intermediates of photounstable filter substances get in direct contact with epidermal and dermal structures (Schallreuter *et al*, 1996; Gulston and Knowland, 1999). Furthermore, Hayden *et al* (1997) recovered oxybenzone as unchanged oxybenzone and metabolites in the urine after topical application. From this point of view it is surprising that at present standardized photostability tests of finished suncare products are not required before marketing, although this has been recommended repeatedly (Berset *et al*, 1996; Vanquerp *et al*, 1998; Sayre and Dowdy, 1999; IARC, 2000b).

MATERIALS AND METHODS

Measurements We purchased 16 commercially available sunscreens (products A–Q) with declared UVA and UVB protection (**Table I**). Nine creams/lotions (products H–Q) were recommended by the producer for use in infancy and childhood. The samples were stored at room temperature and in the dark and opened immediately before our test procedure. By circular movements of a gloved finger quartz glass

slides were covered with a layer of 1.5 ± 0.15 mg per cm² for the standard products (A–G)¹ according to the guidelines for the evaluation of sunscreen products DIN 67501 (Deutsches Institut für Normung) and 2.0 ± 0.2 mg per cm² for child products (H–Q) according to the COLIPA (European Cosmetic, Toiletry and Perfumery Association) guidelines to measure the sun protection factor (SPF) of sunscreens. The correct quantity was checked immediately after application by a laboratory balance. The samples were dried for 30 min at constant temperature (26°C) and constant relative humidity (50%). Thereafter, the slides were irradiated with a solar simulator (COLIPA DermaSun Dr Hönle 400F/5, Planegg, Germany) at a radiometrically (Solar Light SL 5D, Solar Light, Philadelphia, PA) defined, homogeneous field of irradiance. By using the standard erythema dose (SED) (CIE, 1998), the mean biologically effective irradiance was 12.75 SED per h, corresponding to solar radiation at midsummer noon and cloudless sky in central Europe. The variability of the radiation field was 5.3%, which is significantly below the limit of the COLIPA guidelines. During the entire irradiation time the temperature and humidity were kept constant at 26°C and 50%, respectively. The spectral irradiance of the solar simulator was measured by using a spectroradiometer with a double monochromator (Bentham, DTM 300, Bentham, Reading, U.K.) and a photomultiplier (Bentham, DH-1 (Bi), Bentham, Reading, UK) fulfilling

¹Maier H, Brunnhofer K, Schauburger G, Hönigsmann H: Photoinactivation of sun protection products. *Arch Dermatol Res* 290:34, 1998 (abstr.)

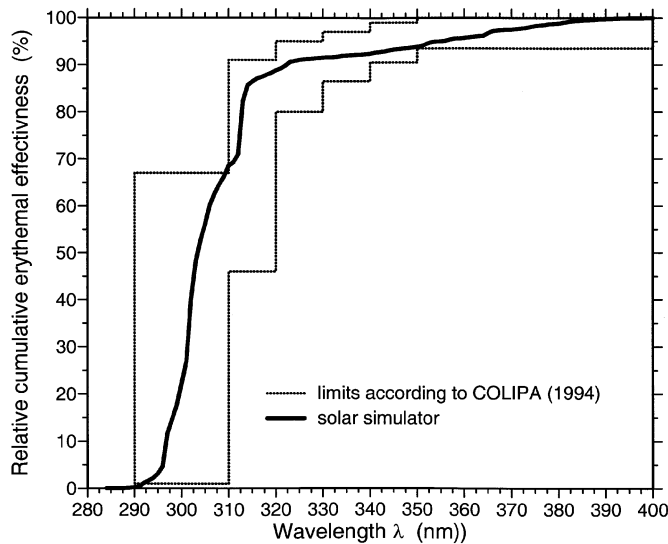


Figure 1. Relative spectral erythral effective irradiance of the solar simulator. The cumulative erythral effectiveness of the simulator lies between the limits according to the COLIPA (1994) guidelines.

the requirements of the COLIPA guidelines (Fig 1). The first series of sunscreen products (A–G) was exposed up to 50 SED, the second series recommended for children (H–Q) was exposed up to 25 SED.

We measured the spectral absorbance (ratio of the absorbed radiant flux to the incident flux according to the CIE International Lighting Vocabulary; CIE, 1987) (in percentage) for the 16 sunscreens in both, the UVA (320–380 nm) and UVB (280–320 nm) range before, after 5 SED (products A, C, E, H–Q), 12.5 and 25 SED (all products), and 50 SED (products A–G) of solar-simulated UV radiation with a resolution of 1 nm by a spectrophotometer (Varian Cary 3E, Varian Australia PTY Ltd, Mulgrave, Victoria, Australia) connected with a sphere (Labsphere DRA-CA-30, Labsphere, North Sutton, NH) (Marginean *et al*, 1995). This instrument is a double-beam, ratio-recording spectrophotometer that automatically performs a baseline correction. To cut off fluorescent effects the sphere was armed with a Schott UG 11 filter (Schott, Mainz, Germany) according to the Australian/New Zealand Standard AS/NZS 4399 (1996). In order to keep the samples in an identical position during consecutive measurements we added a steel frame (inner measurements 25 × 80 mm) to the provided transmittance sample holder. The quartz glass slides were put into the tight fitting frame and fixed by a shutter. An aperture faded out the lateral portions of the sample and allowed transmission only in a central field of approximately 6 cm². The sample holder was screwed up in the transmittance sample port. Two samples of each sunscreen product of the same lot are stored under standardized conditions.

Data analyses In order to describe the effect of photoinstability we used the spectral photoinactivation, ΔA_e , due to the UV exposure, calculated by the difference between the spectral absorbance, $A_{e,D}$, for a UV dose, D, and the spectral absorption before the UV exposure, $A_{e,0}$:

$$\Delta A_{\lambda,D} = A_{\lambda,0} - A_{\lambda,D} \quad (1)$$

The mean difference of the absorbance, ΔA , was calculated for both, the UVA (320–380 nm) and UVB (280–320 nm) range, called as photoinactivation. The results are presented in Table II showing the absorbance before UV exposure, A_0 , the photoinactivation for UVB and UVA, ΔA , as the difference between the absorption before and after UV exposure for following UV doses, D, of 5 SED (products A, C, E, H–Q), 12.5 SED and 25 SED (all products), and 50 SED (products A–G), respectively.

RESULTS

Characteristics of the investigated sunscreens Table I shows the detailed photobiologically relevant data of the selected sunscreen products. The product information printed on the packages of the respective sunscreens promised broad-spectrum UV

Table II. Absorbance^a of the sunscreen products (A–Q)^b before UV exposure and photoinactivation^c after increasing UV exposure^d

Product	Absorbance A (%)	Photoinactivation, ΔA (%), by biologically effective UV-exposure (SED)			
		5	12.5	25	50
UVB					
A	99.6	0.1	0.2	0.2	0.2
B	100.0		0.0	0.0	0.0
C	98.6	0.6	0.7	0.8	1.8
D	99.6		0.0	0.2	0.2
E	99.6	0.2	0.2	0.2	0.4
F	71.3		4.0	−5.1	−1.0
G	99.9		0.0	0.0	0.0
H	99.9	0.0	0.0	0.0	
I	99.8	0.2	0.1	0.1	
K	99.9	0.0	0.0	0.0	
L	99.9	0.0	0.0	0.0	
M	99.9	0.0	0.0	0.0	
N	99.7	0.1	0.1	0.1	
O	99.9	0.0	0.0	0.0	
P	99.9	0.0	0.0	0.0	
Q	99.7	0.3	0.2	0.4	
UVA					
A	98.5	0.3	0.6	0.3	1.6
B	99.4		15.7	28.1	29.7
C	92.6	13.0	36.3	48.4	52.0
D	99.6		0.0	0.2	0.3
E	97.7	5.3	13.8	26.5	32.7
F	57.2		11.7	12.3	27.4
G	70.9		−1.6	−2.0	−3.0
H	97.2	0.0	0.0	0.2	
I	98.4	7.7	13.2	24.3	
K	99.9	0.1	0.0	0.0	
L	99.9	0.0	0.0	0.0	
M	99.9	0.0	0.1	0.5	
N	99.6	0.1	0.1	0.2	
O	99.9	0.0	0.1	0.6	
P	96.0	17.6	23.6	22.6	
Q	95.5	8.3	18.2	31.8	

^aData presented as mean values for the UVB (280–320 nm) and UVA (320–380 nm) range.

^bPhotounstable products are highlighted bold.

^cData presented as mean values for the UVB (280–320 nm) and UVA (320–380 nm) range. The photoinactivation is defined as the change of absorbance due to UV exposure according to Eqn 1.

^dUV exposure is given in standard erythema dose (SED).

protection. For all sunscreen products the SPF (median 20, 16–26) was declared, whereas a quantification of the protective capacity in the UVA range was given for only two (D, N). The term “photostability” was mentioned in the product information of only two sunscreens (A, L) produced by the same company. A detailed list of active ingredients was available for all sunscreens.

Table I shows that the UV broad spectrum protective capacity of the 16 examined sunscreens was due to a total of different filter combinations. None of the examined sunscreen products contained p-aminobenzoic acid (PABA) or PABA derivatives, e.g., octyl dimethyl PABA (padimate-O), or isopropylidibenzoylmethane. Benzophenone-3 (oxybenzone), however, was included in one (product G) and butyl methoxydibenzoylmethane in 14 (A–F, I–Q) of the 16 sunscreens. In all sunscreens except product C and F organic UV filters were combined with one inorganic UV filter, zinc oxide (ZnO) in product H or titanium dioxide (TiO₂) in sunscreen products A, B, D–G, and I–Q.

Wavelength-dependent change of the absorbance The photoinactivation, ΔA , for all measured doses, D, are presented for the UVA and UVB range (Table II).

UVB In the UVB range the absorbance before UV exposure, A_0 , for all products except product F showed values above 98% (Table II). Sunscreen F had a high water content. Owing to the rapid and significant water loss during application it was difficult to achieve a homogeneous layer. This seems to be the reason why the absorbance before UV exposure, A_0 , for product F was only 71%. In the UVB range, the values of the decrease of absorbance (photoinactivation), ΔA , for all sunscreens and UV doses were below 5%.

UVA In the UVA range 14 of 16 products showed an absorbance before UV exposure, A_0 , of more than 90% (Table II). Only for two products we measured an absorbance before UV exposure of less than 90% (product F 57%, product G 71%). Contrary to the behavior in the UVB range seven of 16 products were significantly photounstable in the UVA range, which resulted in a decrease of the absorbance (photoinactivation), ΔA , between 12% (F) and 48% (C) at 25 SED (Table II). The photoinactivation for the nine photostable products, however, was negligible ($< 1\%$). Figure 2 shows the spectral absorbance, A_λ , of two selected products: photostable sunscreen D (Fig 2a) and photounstable sunscreen C (Fig 2b). For photostable product D there was nearly no photoinactivation for both, the UVA and UVB range and for all UV doses (Table II). Product C was selected by the highest values of photoinactivation for the UVA range with increasing UV doses (Table II). The ability of sunscreen product C to absorb UVB, however, was not affected. Figure 3 shows the spectral photoinactivation, ΔA_λ , at a UV exposure of 25 SED for all photounstable products. With the exception of product C and F, these products had a similar spectral behavior. As was discussed earlier the reason for the distinct lower UVB absorbance of product F appears to be its high water content. The decrease of the absorbance for product C and F started at a lower wavelength (about 320 nm) compared with all other photounstable products (about 340 nm). All products except product F showed an increasing photoinactivation with the wavelength. Only product F had a maximum at 365 nm.

Dose-dependent change of the absorbance Table II shows the dose-dependent decrease of the absorbance in the UVA range of the seven photounstable sunscreen products. In order to depict the photoinactivation as a function of wavelength and UV dose, a contour plot was drawn for photounstable sunscreen C (Fig 4). The lowest contour line of 5% indicates that photoinactivation started between 320 nm and 330 nm. Above 360 nm, and for UV doses over 20 SED the photoinactivation reached a maximum with values above 75%. The small distances between the contour lines (between 25% and 55% in the wavelength range between 360 nm and 380 nm) indicates that photochemical inactivation was most significant in this area.

DISCUSSION

Sunscreens have to fulfill a lot of quality criteria before marketing (Shaath, 1997a). Surprisingly, a standardized assessment of photostability of sunscreen products is not required, although it is well known that several UV filter substances are inactivated by UV radiation (Chignell *et al.*, 1980; Gasparro, 1985; Knowland *et al.*, 1993; Schwack and Rudolph, 1995; Allen *et al.*, 1996; Berset *et al.*, 1996; Schallreuter *et al.*, 1996; Vanquerp *et al.*, 1998; Tarras-Wahlberg *et al.*, 1999). This process is dose-dependent (Gasparro, 1985; Schwack and Rudolph, 1995; Allen *et al.*, 1996; Berset *et al.*, 1996; Tarras-Wahlberg *et al.*, 1999) and it appears that the most pronounced photodegradation is induced already by low UV doses (Gasparro, 1985; Berset *et al.*, 1996; Tarras-Wahlberg *et al.*, 1999). Photodecomposition of UV filters results in a change of absorptive capacity (Gasparro, 1985; Berset *et al.*, 1995; Tarras-Wahlberg *et al.*, 1999) and the development of photoproducts (Gasparro, 1985; Schwack and Rudolph, 1995), which might be of biologic relevance when sunscreens are applied as thin layers on human skin (Deflandre and Lang, 1988; Schwack and Rudolph, 1995).

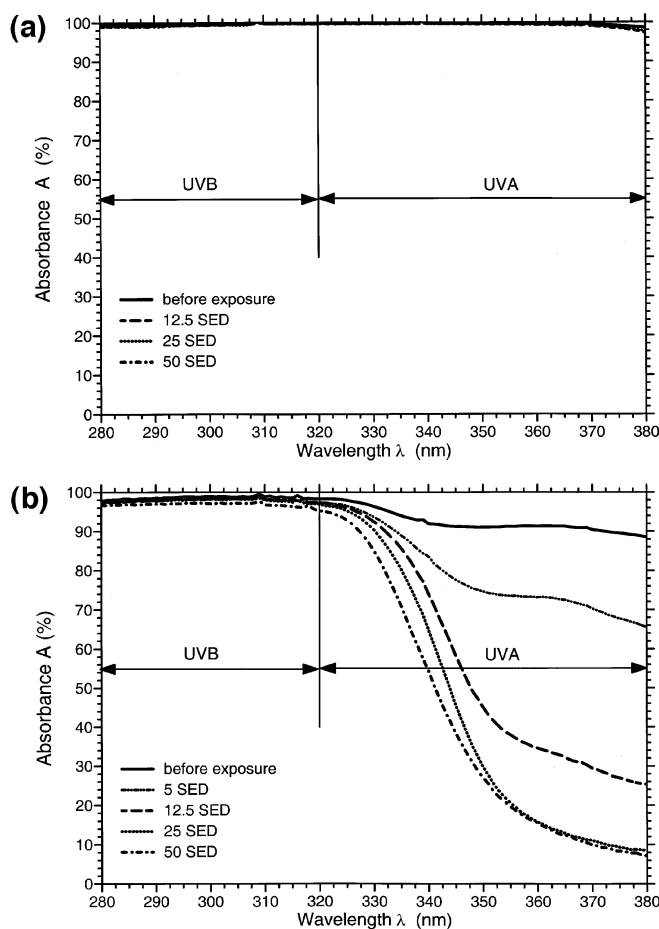


Figure 2. Spectral absorbance, A_λ in %, of photostable product D (a) and photounstable product C (b).

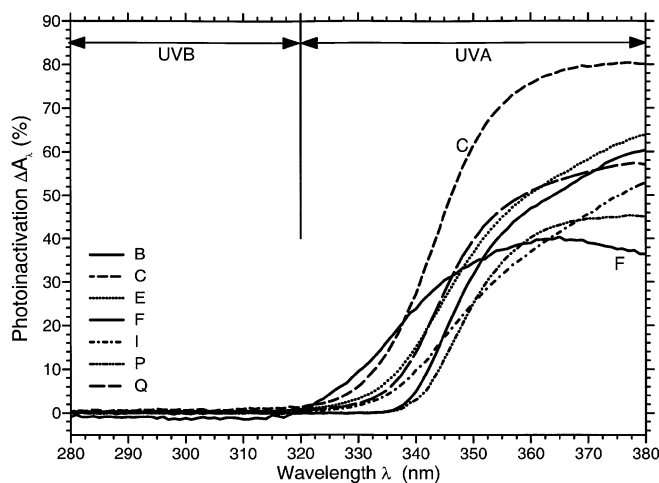


Figure 3. Spectral photoinactivation, ΔA_λ in %, of all photounstable products (B, C, E, F, I, P, and Q) after a UV exposure of 25 SED.

The most recent data on photostability are available for oxybenzone (benzophenone-3), which is a UVA filter substance frequently used in sunscreen products (Schallreuter *et al.*, 1996; Hayden *et al.*, 1997).

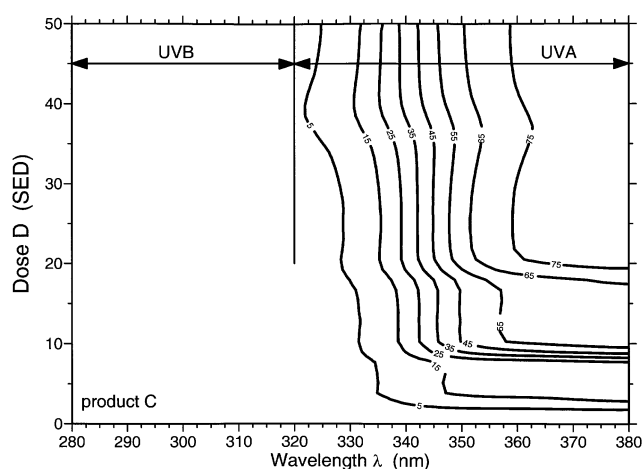


Figure 4. Contour plot of the photoinactivation, $\Delta A_{\lambda,D}$, as a function of the UV dose, D , and the wavelength, λ , for photounstable product C showing a distinct photoinstability.

Schallreuter *et al* (1996) demonstrated that oxybenzone was photo-oxidized to its semiquinone by solar radiation *in vitro* and *in vivo*. This reactive intermediate bound to thiolate groups in the epidermis and inactivated the important anti-oxidant enzyme, thioredoxin reductase. Opposite to these results (Schallreuter *et al*, 1996) a spectroscopic analysis of UV filters (Tarras-Wahlberg *et al*, 1999) failed to show a significant photoinactivation of oxybenzone. The reason for this disagreement may be the fact that oxybenzone in the study by Tarras-Wahlberg *et al* (1999) was dissolved in a nonpolar medium (petrolatum), whereas Schallreuter *et al* (1996) used a sunscreen product (Soltan facial cream) where oxybenzone was distributed in the aqueous phase. The importance of the solvent for the behavior of a UV filter (Agrapidis-Paloympis *et al*, 1987; Shaath, 1997b) is illustrated by a high-performance liquid chromatography analysis by Schwack and Rudolph (1995) which indicated that dibenzoylmethane filters in model solutions were photodegraded in nonpolar solvents but were photostable in polar solvents. Photostability tests of individual filters, however, do not take into account possible interactions of ingredients of complex finished sunscreens (Sayre and Dowdy, 1999). Apart from the solvent problem the photodegradation rate may be affected by presence/absence of oxygen (Schwack and Rudolph, 1995) and recently, Sayre and Dowdy (1999) hypothesized that photosensitivity reactions induced by butyl methoxydibenzoylmethane may be responsible for photodecomposition of certain UVB filters. Another unfavorable interaction of sunscreen ingredients was reported by Bonda and Steinberg (2000). The UV filter ethylhexyl methoxycinnamate significantly reduced the photostabilizing effect of the new photostabilizer diethylhexyl naphthalate on butyl methoxydibenzoylmethane-containing sunscreens.

The influence of the solvent and possible interactions of sunscreen ingredients were the reasons why we evaluated the photostability of finished sunscreen products, which has been recommended by several authors and the IARC Working Group (Berset *et al*, 1996; Vanquerp *et al*, 1998; Sayre and Dowdy, 1999; IARC, 2000b). In our test series seven of 16 sunscreen products showed a significant dose- and wavelength-dependent decrease of the absorbance (photoinactivation) in the UVA range. The decrease of absorbance in the UVA range started between 320 nm and 335 nm with a maximum above 350 nm. Neither complex combinations of different organic filters nor the addition of inorganic filters could absolutely prevent photoinactivation. All photounstable products contained three or five organic filters and all but products C and F contained an inorganic filter. Our data clearly show that the behavior of a complete product when exposed to UV radiation could not be predicted from the behavior of the

individual filter substances it was composed of. In contrast to the photounstable sunscreens B, I, and Q, sunscreen product M was not photoinactivated, although all of them had identical filters. The same was true for photounstable product E that contained the same filter combination as the photostable sunscreens K and O. Furthermore, the presence of a photounstable UV filter among the list of ingredients of a certain sunscreen did not necessarily result in photoinstability of the complete sunscreen product. This could be shown for products that contained butyl methoxydibenzoylmethane or oxybenzone. In our test series only photostable sunscreen G contained oxybenzone and butyl methoxydibenzoylmethane was found in both, photostable (A, D, K-O) and photounstable sunscreens (B, C, E, F, I, P, Q). Two recent studies (Diffey *et al*, 1997; Sayre and Dowdy, 1999) reported comparable results. Diffey *et al* (1997) showed that in a series of nine products only four sunscreens were photostable. Sayre and Dowdy (1999) presented data on dose-dependent photodegradation of six butyl methoxydibenzoylmethane-containing sunscreens. Both studies (Diffey *et al*, 1997; Sayre and Dowdy, 1999) are in agreement with our observation that photoinactivation was most pronounced in the wavelength range above 350 nm and after the first fraction of UV exposure (Table II).

Photounstable UV filters may damage human skin by two mechanisms which, at the end, are closely related and most likely boost each other. First, UV filters may behave as exogenous UVA sensitizers. During photolysis of photounstable UV filters reactive intermediates are produced (Chignell *et al*, 1980; Gasparro, 1985; Dalle Carbonare and Pathak, 1992; Schwack and Rudolph, 1995; Allen *et al*, 1996; Schallreuter *et al*, 1996; Tarras-Wahlberg *et al*, 1999). Free radicals may induce formation of reactive oxygen species (ROS) (Dalle Carbonare and Pathak, 1992; Allen *et al*, 1996) which have toxic effects (Dalle Carbonare and Pathak, 1992; Shindo *et al*, 1994; Schallreuter *et al*, 1996; McHugh and Knowland, 1997) or may bind to proteins or DNA (Dalle Carbonare and Pathak, 1992). Second, a dose-dependent decrease of the UVA absorptive capacity results in an increase of the direct UVA-induced skin damage.

According to the free radical theory of photoaging of human skin generation of ROS results in the formation of protein cross-links in collagen and certain enzymes such as catalase and superoxide dismutase (Dalle Carbonare and Pathak, 1992). These authors showed that protein inactivation was more significant by UVA irradiation in the presence of sensitizers than by UVA without sensitizers. There is increasing evidence that oxidative stress is involved in skin carcinogenesis. ROS seem to be responsible for the induction of 8-hydroxy-2'-deguanosin in arsenic-related nonmelanoma skin cancer (Matsui *et al*, 1999). The presence of 8-hydroxy-2'-deguanosin-mediated DNA defects in UV-induced skin cancers in mice (Nishigori *et al*, 1994) indicated ROS as cofactors of photocarcinogenesis. This seems to be the mechanism for the significantly higher number of strandbreaks of single-stranded DNA by UV irradiation in the presence of octyl dimethyl PABA (padimate-O) when compared with UV irradiation alone (McHugh and Knowland, 1997). In basal keratinocytes padimate-O significantly increased indirect DNA damage (strand breaks) when exposed to UV radiation even though the sunscreen film reduced direct UV damage (Gulston and Knowland, 1999). This indirect (ROS-mediated) DNA damage could be suppressed completely by oxygen quenchers. The study by Gulston and Knowland (1999), therefore, strongly supports the model of a dual mechanism of UVA-induced skin damage. Surprisingly, padimate-O, which penetrates the skin (Kenney *et al*, 1995), is still advertised as a chemically inert, safe, and photostable UVB filter (Klein, 1997). Photoinstability of UV filters may be responsible for the high frequency of photosensitive reactions induced by sunscreens. The hypothesis that reactive photolysis products may behave as haptens or induce toxic cell damage (Chignell *et al*, 1980) is supported by the results of three recent studies presenting the patch and photopatch test results in persons with suspected photosensitivity to sunscreen ingredients (Szczerko *et al*, 1994; Schauder and

Ippen, 1997; Journe *et al*, 1999). In their review of 402 patients Schauder and Ippen (1997) found that UVA filters were responsible for the majority of positive photopatch test results (54 reactions) when compared with UVB filters (30 reactions). The photo-unstable absorbers isopropylidibenzoylmethane and butyl methoxydibenzoylmethane gave 32 and 13 positive reactions, respectively. Photostable terephthalylidene dicamphor sulfonic acid, however, showed no positive reactions in this follow-up. The most important photosensitizers in the survey by Szczurko *et al* (1994) were oxybenzone, octyl dimethyl PABA and isopropylidibenzoylmethane and in the follow-up by Journe *et al* (1999) oxybenzone and isopropyl dibenzoylmethane.

Until recently, microfine inorganic filters seemed to be ideal ingredients to provide broad-spectrum UV protection and to maintain photostability of sunscreens (Anderson *et al*, 1997; Mitchnick *et al*, 1999). Dunford *et al* (1997), however, showed that microfine ZnO and TiO₂ not only scattered and reflected but effectively absorbed UV radiation thus initiating ROS formation and catalyzing DNA damage. For uncoated ZnO and for both, the coated and uncoated TiO₂ Mitchnick *et al* (1999) measured a significant photocatalytic potential (ZnO < TiO₂), whereas the photoreactivity of coated microfine ZnO appeared to be negligible. As long as relevant transepidermal penetration of microparticles cannot be excluded definitely (Lademann *et al*, 1999) the photocatalytic potential of inorganic sunscreen ingredients is of importance and should be further investigated (IARC, 2000b).

It is well known that suberythemogenic UVA doses do cause various biologic effects (Tyrrell, 1995). UVA induced nonmelanoma skin cancer in SKH1 hairless albino mice (de Gruijl *et al*, 1993), although it was far less effective than UVB. The p53 expression induced by UVA was only half of that induced by biologically equivalent doses of solar-simulated radiation, pyrimidine dimer formation, however, was the same (Burren *et al*, 1998). Setlow *et al* (1993) defined an action spectrum for fish melanoma with a carcinogenic peak at 365 nm wavelength in the UVA range. The importance of UVA radiation for the development of melanoma in humans, however, is not clear as yet. Several studies agree that UVA appears to participate in the photoaging process more effectively than originally suspected (Menter *et al*, 1996; Berneburg *et al*, 1999). The effect of UVA on the immune system, however, is still a matter for debate. Cowell *et al*² showed that solar radiation or solar-simulated radiation had a higher immunosuppressive potential than UVB radiation. This is the reason why Serre *et al* (1997) proposed that only sunscreens with medium SPF and high UVA protection adequately protect from photoimmunosuppression. Recent studies questioned the hypothesis of an immunosuppressive action of UVA (Halliday *et al*, 1998; Iwai *et al*, 1999) and indicated that UVA may provide some protection against UVB-induced immunosuppression (Reeve *et al*, 1998, 1999; Reeve and Tyrrell, 1999). Direct UV damage of nonenzymatic and enzymatic anti-oxidants (Dalle Carbonare and Pathak, 1992; Shindo *et al*, 1994; Schallreuter *et al*, 1996; Poswig *et al*, 1999) should not be underestimated as a causative factor for skin damage but in the case of superoxide dismutase appears to be dependent on the irradiation mode (Shindo *et al*, 1994; Poswig *et al*, 1999).

As was shown, the behavior of a sunscreen could not be predicted from the behavior of its individual ingredients. Neither the combination of various organic filters nor the addition of inorganic filters were able to guarantee photostability. What is most striking is that photoinactivation was not a phenomenon of high UV doses but appears to take place at doses that can easily be acquired by sunbathers. We therefore conclude that only photostable organic filters should be used and that the safety of inorganic UV filter substances must be re-evaluated. A standardized assess-

ment of the photostability of suncare products should be a general requirement before marketing.

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